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Circulating Insulin-Like Growth Factor I, Insulin-Like Growth Factor Binding Proteins, Growth Hormone, and Resumption of Estrus in Postpartum Cows Subjected to Dietary Energy Restriction[1]

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ABSTRACT: The objective of this study was to determine whether serum concentrations of growth hormone (**GH**), IGF-I, IGF binding proteins (IGFBP), and glucose at wk 2 and 10 postpartum were associated with the ability of postpartum beef cows to resume cycling when maintained on a limited nutrient environment. Cows (n = 29) were individually fed either 130 or 170 kcal ME × $BW^{-.75}$ × d^{-1} during nonlactation and 170 or 210 kcal ME × $BW^{-.75}$ × d^{-1} during lactation for an average of 4.1 yr before sample collection. The proportion of cows that resumed estrus within 20 wk after parturition was less ($P < .05$) at the lower feeding rate (5 of 14) than at the higher feeding rate (11 of 15). Concentrations of IGF-I increased from wk 2 to 10 in cows that resumed cycling but not in cows that remained anestrous and were less ($P < .05$) at wk 2 and 10 in cows that remained anestrous compared to cows that resumed cycling. Circulating amounts of IGFBP-2 at wk 2 were greater ($P < .05$) and IGFBP-3 concentrations were lower ($P < .05$) in cows that remained anestrous compared to cows that resumed cycling. Cows on the lower feeding rate that did not cycle had lower body condition scores and greater concentrations of GH compared ($P < .05$) to other cows. At the higher feeding rate, body condition score and concentrations of GH did not differ between cows that did or did not resume cycling. Circulating concentrations of IGF-I and IGFBP-2 and -3 at wk 2 postpartum were indicators of the capacity of energy-restricted cattle to resume cycling after parturition.

Key Words: Cattle, Somatotropin, Insulin-Like Growth Factor, Anestrus, Postpartum Period

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Introduction

The adverse effects of dietary energy restriction on reproductive function in cattle have been reviewed extensively (Randel, 1990; Short et al., 1990; Williams, 1990; Dunn and Moss, 1992; Schillo, 1992). Research cited in these reviews indicates that restricted energy intake suppresses hypothalamic secretion of LHRH. However, the mechanism(s) by which restricted energy intake suppresses LHRH secretion has not been determined. In cattle and other animals, alterations of the growth hormone (**GH**):IGF:IGF binding protein (**IGFBP**) axis occur in response to nutritional stress (McGuire et al., 1992; Thissen et al., 1994). An underlying hypothesis of the present research is that alterations in the GH:IGF:IGFBP axis may provide a mechanism by which the hypothalamus perceives changes in metabolic status. Identification of endocrine or metabolic factors associated with the nutritional influences on reproductive function is expected to facilitate the ability of producers to select females most suited for particular nutritional environments. The objective of the present study was to determine whether circulating concentrations of GH, IGF-I, IGFBP, and glucose are associated with the ability of postpartum beef cattle to resume cycling when maintained in a limited nutrient environment.

Materials and Methods

Animals

Cows (n = 29) evaluated in this study were a subset of animals from a comprehensive project

[1]Mention of trade names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. The authors express their gratitude to Pat Nuss, Sheila Schemm, and Jodi Schulte for technical assistance and to Linda Parnell for secretarial assistance.

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evaluating life-cycle production efficiency of nine breeds maintained on four different levels of energy intake (Nugent et al., 1993; Jenkins and Ferrell, 1994). Detailed descriptions of animal populations, feeding, management, and production records are provided within the above references. Cows in this study were selected from the two lowest feeding levels, which were 58 (n = 14) and 76 (n = 15) g of DMI × initial $BW^{-.75}$ × d^{-1} during nonlactation. Feeding levels were increased in both groups by 18 g of DMI × $BW^{-.75}$ × d^{-1} during lactation. Cows were on these feeding levels for an average of 4.1 yr before they were evaluated in the present study. The cows' initial body weight was determined before they entered the life-cycle production efficiency project when they (5 to 8 yr of age) were in their 6th to 7th mo of gestation. The diet consisted of 77.5% ground alfalfa, 17.5% corn, and 5% corn silage (CP = 16%; ME = 2.25 Mcal/kg as calculated from tabular values in NRC, 1984). Cows were individually fed in bunks equipped with electronic head gates (American Calan, Northwood, NH). Based on the diet and amount fed, cows received either 130 or 170 kcal ME × initial $BW^{-.75}$ × d^{-1} during nonlactation and 170 or 210 kcal ME × initial $BW^{-.75}$ × d^{-1} during lactation. Breeds included Braunvieh, Charolais, Hereford, and Red Poll (n = 3 to 5 cows/breed in each feeding level).

At wk 2 and 10 postpartum, body condition scores (9-point scale) were estimated for each cow, and animals were fitted with indwelling jugular catheters. Blood samples (10 mL) were collected at hourly intervals for 4 h, before feeding on the day after catheterization. Hourly samples for the determination of glucose concentrations were collected into 2.7-mL Monovette tubes containing 2.7 mg of fluoride and 3.24 mg of EDTA (Starstedt, Arlington Heights, IL). Coccygeal venipuncture samples were collected from every cow at weekly intervals from wk 3 to 20 postpartum or until resumption of estrous activity had been verified prior to wk 20. All blood samples were placed on ice immediately after collection. Samples collected for glucose analysis were centrifuged within 15 min after collection and plasma was collected and stored at –20°C. All other samples were allowed to clot overnight then centrifuged at 1,500 × *g*, and serum was harvested and stored at –20°C until it was analyzed as described below. For the purpose of this study, the week in which estrous activity had resumed was defined as the week prior to the first time circulating concentrations of progesterone exceeded 1 ng/mL for two consecutive weeks, as previously described (Nugent et al., 1993).

Hormone and Insulin-Like Growth Factor Binding Protein Analyses. Serum from samples collected at weekly intervals was analyzed by RIA to determine progesterone concentrations as previously described (Roberson et al., 1989). Samples were analyzed in four assays performed at approximately monthly intervals to allow animals that had progesterone profiles indicative of normal luteal activity to be removed from the weekly bleeding schedule. Intra- and interassay CV were 3.1 and 13.8%, respectively.

Circulating concentrations of GH in hourly samples collected at wk 2 and 10 postpartum were determined by RIA as previously described using USDA-bGH-B1 as the reference standard (AFP-5200), NIDDK-oGH-I-4 (AFP-8758C) for the iodination preparation, and NIAMDD-anti-oGH-2 as the primary antisera (Klindt et al., 1985; the antisera and hormone for iodination were provided by the USDA hormone program). Intra- and interassay CV were 14.4 and 9.1%, respectively.

Circulating concentrations of IGF-I in a composite of hourly serum samples collected from each cow at wk 2 and 10 postpartum were determined in one RIA (CV = 9.9%) after acid-ethanol extraction of IGFBP from serum as previously described (Funston et al., 1995). The antiserum (UB3-189) used in this RIA was generously provided by the National Hormone and Pituitary Program.

Concentrations of glucose were determined with an automated glucose oxidase method using the Technicon AutoAnalyzer II Method #339-19 (Technicon Industrial Systems, Tarrytown, NY).

Relative abundance of IGFBP in a composite of hourly serum samples collected at wk 2 and 10 from each cow was determined by ligand blot analysis (Hossenlopp et al., 1986) as described previously (Funston et al., 1995). Samples (1 μL of serum) were subjected to one-dimensional SDS-PAGE under non-reducing conditions (Laemmli, 1970). Proteins were electrophoretically transferred to nitrocellulose membranes that were subsequently probed with iodinated IGF-I. Probed membranes were exposed (5 and 14 d) to autoradiographic films, and binding intensities of IGFBP were analyzed with an LKB Bromma UltroScan XL Laser Densitometer equipped with the Gel Scan XL software (Pharmacia LKB, Uppsala, Sweden). The gel (Hoefer SE 600, Hoefer Scientific Instruments, San Francisco, CA) and transfer (Hoefer Transpor II) apparatuses used in this procedure were of sufficient capacity to handle four gels, with 15 lanes per gel, at one time so all samples could be analyzed simultaneously.

Identification of IGFBP in serum was determined by immunoprecipitation with antiserum against specific IGFBP and subjecting the resulting precipitates to ligand blot analysis. The antisera used included rabbit anti-rat IGFBP-1 (generously provided by N. Ling, The Whittier Institute for Diabetes and Endocrinology, Scripps Memorial Hospital, LaJolla, CA), rabbit anti-human IGFBP-1, rabbit anti-bovine IGFBP-2, rabbit anti-human IGFBP-4, and rabbit anti-human IGFBP-5 (all four from Upstate Biotechnology, Lake Placid, NY). Immunoprecipitation was accomplished as previously described (Funston et al., 1996). Briefly, 2 μL of serum was combined with 1 μL of antiserum and 45 μL of buffer (50 m*M* Tris, 3 *M* NaCl, 1 m*M* EDTA, 2%

[vol/vol] Triton X-100, and .02% [wt/vol] NaN_3, pH 7.4). Samples were incubated overnight at 4°C while rotating on a ROTO-TORQUE (Cole Parmer Instrument, Chicago, IL) to provide continuous mixing. The following morning, 20 μL of preprecipitated goat anti-rabbit gamma globulin was added to each sample. Samples were incubated an additional 3 h at 4°C with rotation and then centrifuged at approximately 12,000 $\times$ *g* for 10 min. The resulting precipitate from each sample was washed twice by resuspension in .4 mL of the buffer described above and subsequent centrifugation. Precipitates were redissolved in 40 μL of gel loading buffer and subjected to ligand blot analysis following SDS-PAGE as described. A minimum of three serum samples were evaluated with each IGFBP antiserum.

Statistical Analyses. Chi-square analysis was used to determine whether differences in the proportion of cows that resumed cycling existed between the two rates of ME intake. In animals that did resume cycling, the effect of diet (i.e., rate of ME intake) on the week that resumption of estrus occurred was analyzed by ANOVA for a completely random design using the GLM procedure of SAS (1985). Because not all animals resumed cycling during the 20-wk postpartum period, cows were classified by dietary treatment and whether they resumed cycling during the 20-wk period (estrus status). Thus, estrus status was analyzed as a discrete variable and actual week that estrus resumed was not considered. Differences in body condition scores and serum concentrations of IGF-I, GH, glucose, and IGFBP among classification groups and week of sampling were analyzed by ANOVA for a split-plot design using the GLM procedure of SAS (1985). The model included diet, estrus status (i.e., did or did not resume cycling by wk 20 postpartum), the interaction of diet $\times$ estrus status, cow within diet $\times$ estrus status (used as the error term to test the effects of the previous classification variables), week of sampling and the interactions of diet $\times$ week, estrus status $\times$ week, and diet $\times$ estrus status $\times$ week. Least squares means for significant effects observed in the ANOVA were separated with the PDIFF procedure of SAS (1985). Statistical results from data for ligand blot analysis of IGFBP were essentially the same for 5- and 14-d exposures. Therefore, only data from 14-d exposures are presented.

Results

Estrus Status and Level of Dietary Intake. The proportion of cows that resumed ovarian estrous activity within 20 wk after parturition was less (P < .05) at the lower daily feeding rate (5 of 14) than at the higher feeding rate (11 of 15). Among animals that resumed cycling by wk 20, the average weeks to resumption of estrus was 8.0 ± 1.5 and 11.2 ± 1.0 (least squares means ± SE) for the low and high feeding rates, respectively (P = .1). Body condition scores of cows differed (P < .01) due to diet by estrus status classification (see Figure 1) and decreased (P < .06) from wk 2 (4.0 ± .1) to wk 10 (3.7 ± .1).

Circulating Concentrations of Insulin-Like Growth Factor I, Growth Hormone, and Glucose. Circulating concentrations of IGF-I differed due to diet by estrus status classification (P < .05; Figure 1) and due to estrus status by week classification (P < .002; Figure 2). In the diet by estrus status classification, circulating concentrations of IGF-I did not differ (P = .3) between feeding rates for cows that failed to resume cycling. Circulating concentrations of IGF-I in the noncyclic cows were less (P < .01) for the low feeding rate or tended to be less (P = .11) for high feeding rate than those observed in cows on the high feed intake that resumed cycling. Mean concentrations of IGF-I in cows on the low feeding level that resumed cycling were greater (P < .05) than in other classified groups. When classified by estrus status and week, concentrations of IGF-I were less (P < .001) at wk 2 and 10 in cows that failed to cycle than in cows that resumed cycling. Concentrations of IGF-I increased (P < .001) over time in animals that resumed cycling but not (P = .6) in animals that remained anestrous.

Circulating concentrations of GH were also influenced (P < .07) due to diet by estrous status classification. Cows on the low feeding rate that failed to resume cycling had greater (P < .05) levels of GH than cows that resumed cycling. In cows that failed to resume cycling, circulating concentrations of GH tended (P = .1) to be greater in cows on the lower rate of feeding than in cows on the higher feeding rate (Figure 1). Circulating concentrations of glucose differed (P < .001) due to week of sampling (4.8 ± .1 vs 4.1 ± .1 m*M* for wk 2 and wk 10, respectively) but not by other methods of classification examined.

Serum Insulin-Like Growth Factor Binding Proteins. A ligand blot illustrating the IGFBP detected in serum from cows is shown in Figure 3. Previous work indicates that IGFBP-3 migrates as a 40- to 44-kDa doublet (top two bands in lane 1 of Figure 3;

Table 1. Mean (± pooled SD) IGF binding intensity (arbitrary densitometer units) by individual IGF binding proteins (IGFBP)

IGFBP-3	IGFBP-2	31 kDa	28 to 29 kDa	24 kDa
23.1 ± 2.5[a]	8.4 ± 1.2[b]	1.7 ± .9[c]	1.8 ± .4	3.2 ± .4

[a]Lower (P < .05) in animals that failed to cycle (21.4 ± .9) than in animals that resumed cycling (24.5 ± .8).

[b]Differed due to estrus status by week of sampling classification (P < .001); see Figure 2.

[c]Differed due to estrus status by diet classification (P = .1; Figure 1), and binding intensity declined (P < .01) from wk 2 (2.1 ± .2) to wk 10 (1.3 ± .2).

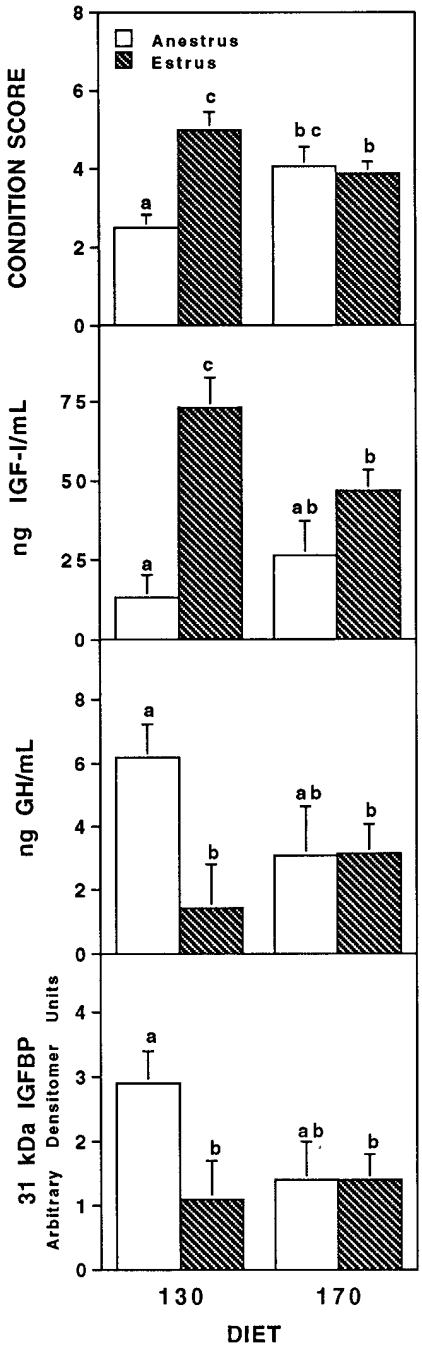

Figure 1. Body condition scores, circulating concentrations of IGF-I and growth hormone (GH), and relative amounts (arbitrary densitometer units) of the 31-kDa IGF binding protein (IGFBP) for cows maintained on two levels of energy intake (130 vs 170 kcal ME $\times$ $BW^{-.75}$ $\times$ d^{-1}) that did (hatched bars) or did not (open bars) resume estrous activity during a 20-wk period after parturition. Values represent the least squares means (± SE) of measurements taken at wk 2 and 10 postpartum. Bars without a common superscript differ ($P < .05$). Concentrations of IGF-I in cows fed the higher level of energy intake tended to differ ($P = .11$) between cows that did or did not resume cycling. For cows that failed to resume cycling, concentrations of GH and 31-kDa IGFBP tended ($P \leq .1$) to differ between levels of energy intake.

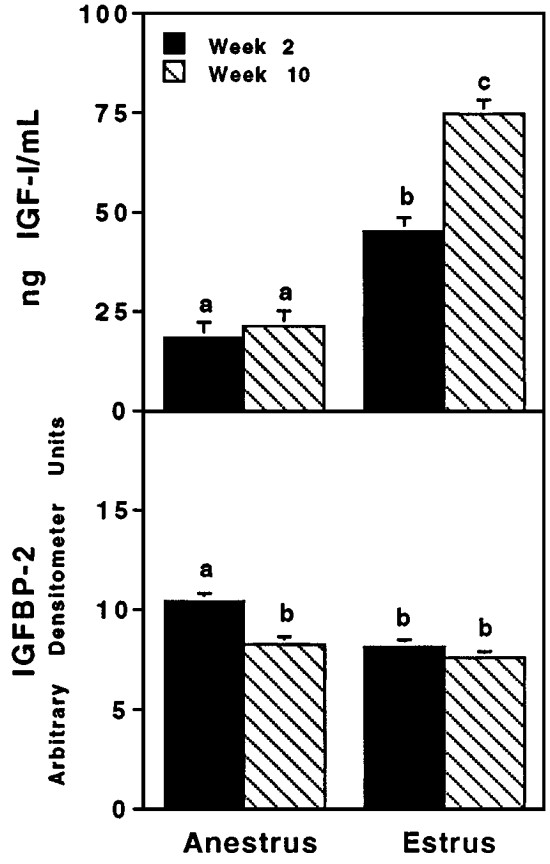

Figure 2. Circulating concentrations of IGF-I and IGF binding protein 2 (IGFBP-2) during wk 2 and 10 postpartum for cows that remained anestrous (Anestrus) or resumed cycling (Estrus) during the 20-wk period after parturition. Values represent least squares means (± SE) for cows from the two levels of ME intake. Bars without a common superscript differ ($P < .001$).

Funston et al., 1995, 1996). Immunoprecipitation analysis identified a 34-kDa protein as IGFBP-2, 24- and 28-kDa proteins as IGFBP-4, and faint bands in the 29- to 31-kDa range as IGFBP-5. Attempts to identify IGFBP-1 in bovine samples by immunoprecipitation with antibodies against human and rat IGFBP-1 were not successful. Mean intensities of IGF-I binding for each IGFBP are shown in Table 1. Circulating amounts of IGFBP-3 were lower ($P < .05$) in animals that failed to cycle than in cows that did resume cycling (see Table 1). Relative binding intensity by the 31-kDa IGFBP differed due to estrus status $\times$ diet classification ($P = .1$; Figure 1). Cows on the lower feeding level that failed to cycle had greater 31-kDa IGFBP activity than cows that resumed cycling ($P < .05$) or cows on the higher level of feeding that failed to cycle ($P < .09$). In addition, a decrease ($P < .01$) in the amount of the 31-kDa IGFBP occurred between wk 2 and 10 (Table 1). Circulating amounts of IGFBP-2 varied due to estrus status by week of sampling classification, being greater ($P <$

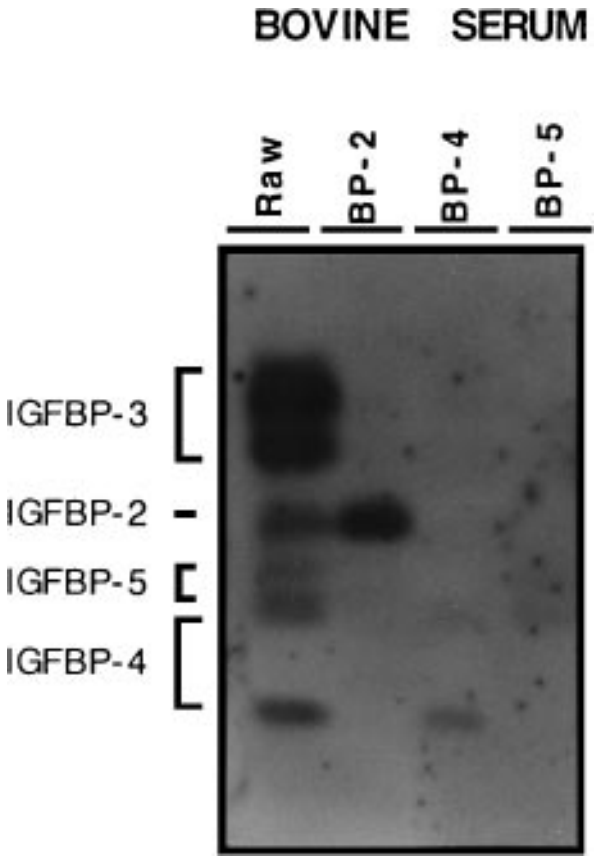

Figure 3. Representative ligand blot of IGF binding proteins (IGFBP) detected in bovine serum before (raw) or after immunoprecipitation with antiserum against IGFBP-2 (BP-2), IGFBP-4 (BP-4), or IGFBP-5 (BP-5) as indicated above each lane. Previous work demonstrated that IGFBP-3 migrated as two glycosylated forms of approximately 40 and 44 kDa (Funston et al., 1995, 1996). Immunoprecipitation resulted in the detection of IGFBP-2 as a 34-kDa protein and IGFBP-4 as 24- and 28- (faintly evident) kDa proteins; IGFBP-5 was scarcely detected as a 29- to 31-kDa doublet.

.001) at wk 2 in cows that failed to resume cycling than in cows that did resume cycling (Figure 2). The 28- and 24-kDa forms of IGFBP-4 did not differ ($P >$ .1) due to level of dietary intake, capacity to resume cycling, or week of sampling.

Discussion

The present study provides evidence that circulating concentrations of certain components of the GH-IGF-IGFBP axis may be indicative of the nutritional competency for reestablishment of ovarian cyclicity during the postpartum period of beef cattle. Circulating concentrations of IGF-I were greater in cows that subsequently resumed cyclicity than in cows that remained anestrous, and concentrations of IGF-I increased between wk 2 and 10 in cows that resumed cycling but not in cows that remained anestrous. Cows that failed to resume cycling also had greater amounts of circulating IGFBP-2 at wk 2 postpartum and decreased levels of IGFBP-3 compared with cows that resumed cycling during the 20-wk period. In addition, levels of GH and the 31-kDa IGFBP tended to be elevated in cows on the low levels of feed intake that failed to resume estrus compared with other animals. Based on these observations, we propose that circulating concentrations of GH, IGF-I, and IGFBP, particularly IGFBP-2,-3, and the 31-kDa IGFBP, may provide a clinical approach for evaluating the nutritional status of individual animals on a within herd basis. Such an approach would have application in the selection of females capable of reestablishing estrus during the postpartum period when managed under particular nutrient environments.

The differences observed for GH, IGF-I, and IGFBP-2, -3, and the 31-kDa IGFBP likely reflect differences in the metabolic status of individual cows, even though the main effect of diet was not significant ($P >$ .14) for these variables. These differences are expected to be due to differences in metabolic efficiency of individuals that would influence their ability to resume cycling during the postpartum period. Under adequate dietary availability, circulating concentrations of IGF-I are under positive regulation by GH (Cohick et al., 1989). In contrast, dietary restriction results in elevated circulating GH, suppressed IGF-I, and a loss of IGF-I responsiveness to exogenous GH treatment (see review by McGuire et al., 1992). The loss of IGF-I responsiveness to GH in dietary restricted cattle may be due in part to decreased hepatic binding sites for GH (Breier et al., 1988). The elevated GH is in turn proposed to be due to a decrease in the negative feedback effect of IGF-I on the hypothalamic-pituitary regulation of GH secretion resulting in increased pituitary synthesis and secretion of GH (Kirby et al., 1993). In the present study, anestrous cows on the low level of ME intake exhibited this classic uncoupling of the GH:IGF-I axis (i.e., GH was increased and IGF-I was decreased). In addition, these cows had lower condition scores than other cows in the study. In contrast, concentrations of GH and condition score were not different between animals on the higher level of ME that did or did not resume cycling, yet concentrations of IGF-I tended to be reduced in the cows that failed to cycle. A possible interpretation of these results is that a loss of IGF-I sensitivity to GH occurred in cows on the higher level of ME intake, but hypothalamic secretion of GH was not affected.

As previously demonstrated, circulating concentrations of IGF-I decline at parturition and gradually increase over time, whereas concentrations of GH increase at parturition and then decline over time (Ronge et al., 1988; Schams et al., 1991; Vicini et al.,

1991). The magnitude in decline and duration of time required for IGF-I levels to return to prepartum levels are greater in animals subjected to dietary restriction (Ronge et al., 1988; Spicer et al., 1990; Nugent et al., 1993; Ryan et al., 1994) and may interact with genetic potential for milk production (Schams et al., 1991). In the present study, concentrations of IGF-I increased over time in cows that resumed cycling but not in cows that remained anestrous, indicating differences in metabolic status during this period that seem to be associated with nutritional influences on reproductive function. Negative correlations between circulating concentrations of IGF-I and duration of postpartum anestrus have been previously reported (Simpson et al., 1992; Nugent et al., 1993). In puerperal cows maintained over a range of body conditions, cows with low condition scores (i.e., ≤ 4) had increased circulating GH, decreased circulating and intrafollicular concentrations of IGF-I, and fewer medium and large ovarian follicles compared to cows with condition scores ≥ 6 (Ryan et al., 1994). In addition, induction of anestrus by nutritional restriction of cycling animals was associated with decreased circulating concentrations of IGF-I and a loss in the normal postcastrational rise in circulating LH (Richards et al., 1991).

The role that IGF-I may have in mediating nutritional effects on reproductive function is further complicated by the existence of high-affinity IGF binding proteins (IGFBP). To date, six IGFBP (IGFBP-1 to -6) have been identified (Shimasaki and Ling, 1991). Even though the biological actions of these IGFBP remain to be fully elucidated, they have been shown to increase the circulating half-lives of IGF (Baxter, 1993) and may inhibit or potentiate the bioavailability of IGF at the cellular level depending on the IGFBP and conditions used for evaluation (Jones and Clemmons, 1995).

Nutritional regulation of IGFBP has been most extensively evaluated in humans and rats (as reviewed in Baxter, 1993; Cohick and Clemmons, 1993; Thissen et al., 1994; Jones and Clemmons, 1995). The majority of research conducted has been limited to IGFBP-1, –2, and –3; much less information is available on IGFBP-4, –5, and –6. In nonruminants, circulating concentrations of IGFBP-1, but not of other IGFBP, fluctuate in response to ingestion of feed. Acute decreases in circulating IGFBP-1 occur in response to increases in insulin or glucose, whereas acute or chronic starvation results in increased circulating IGFBP-1. Studies concerning circulating levels of IGFBP-1 in ruminants are limited (McGuire et al., 1992), primarily due to the inability to identify this protein in samples from ruminants because immunological reagents are not available. Attempts to identify IGFBP-1 in serum of cattle in the present study were also not successful. However, IGFBP-1 may contribute to binding of IGF-I in the 31-kDa range of the ligand blot, because this is similar in size to that reported for ovine IGFBP-1 (Gallaher et al., 1992; Lord et al., 1994). In the present study, the 31-kDa IGFBP was elevated in animals that clearly demonstrated nutritional uncoupling of the GH-IGF axis (i.e., cows on the low feeding rate that failed to cycle).

In ruminants, dietary restriction or periods of negative energy balance are associated with increased circulating IGFBP-2 and decreased IGFBP-3 (Vicini et al., 1991; Gallaher et al., 1992, 1995). Even though level of ME intake did not account for significant variation in IGFBP-2 or -3 in the present study, IGFBP-3 was suppressed and IGFBP-2 was elevated in cows that failed to resume ovarian cyclicity. Mechanisms involved in mediating changes in circulating IGFBP have been associated with nutritionally induced changes in the GH:IGF-I axis. In adequately fed cows, GH administration increased circulating IGF-I and IGFBP-3 but decreased circulating IGFBP-2 (Vicini et al., 1991; Cohick et al., 1992; Stanko et al., 1994). In other studies, circulating IGFBP-3 was unaltered after circulating IGF-I was increased due to exogenous treatment with IGF-I (Cottam et al., 1992) or by protein supplementation (Clarke et al., 1993). Therefore, changes in IGFBP-3 in response to GH treatment may not be mediated by alterations in IGF-I levels. However, administration of GH to dietary-restricted lambs failed to alter the suppressed levels of IGF-I or IGFBP-3 (Hodgkinson et al., 1991). Thus, dietary restriction seems to uncouple the ability of GH to increase circulating levels of IGFBP-3 and IGF-I, consistent with results of the present study.

In contrast to IGFBP-3, administration of IGF-I to adequately fed lambs resulted in decreased circulating levels of IGFBP-2 (Cottam et al., 1992; Gallaher et al., 1995), indicating that GH effects on this protein may be mediated by changes in circulating IGF-I. However, no change in circulating IGF-I was observed when circulating IGFBP-2 was increased in lambs after they were switched from an ad libitum intake to a maintenance diet, but the increase in IGFBP-2 was prevented by administration of IGF-I (Gallaher et al., 1995). In the present study, IGFBP-2 was elevated at wk 2 but not wk 10 in cows that remained anestrous, even though levels of IGF-I were suppressed at both time periods. McGuire and coworkers (1992) demonstrated that alterations in circulating IGF-I due to changes in dietary protein and energy intake were not accompanied by changes in circulating IGFBP-2. Therefore, fluctuations in IGF-I do not seem to be obligatory for changes in IGFBP-2 to occur, and vice versa.

The present study documents the presence of IGFBP-4 as 24- and 28-kDa forms in bovine serum, confirming the recent immunological identification of this protein in bovine sera and follicular fluid (Cohick

et al., 1996; Funston et al., 1996). Amount of IGFBP-4 did not differ among animals maintained on different levels of ME in the present study. Previous research indicates that neither GH treatment of lactating dairy cows (Cohick et al., 1992) nor IGF-I treatment of growing lambs (Cottam et al., 1992) significantly altered circulating levels of IGFBP-4. However, withholding feed for 72 h from lambs, but not ewes, resulted in decreased IGFBP-4 (Gallaher et al., 1992). In addition, suppression of circulating levels of GH and IGF-I by immunization against GH releasing factor resulted in decreased circulating IGFBP-4 in heifers (Cohick et al., 1996). Therefore, nutritional restriction and(or) suppression of the GH-IGF axis may alter IGFBP-4 in young growing animals but not in mature animals. Results from the present study support the hypothesis that chronic nutritional restriction does not alter circulating amounts of IGFBP-4 in adult cows.

Even though the present study provides evidence that circulating IGF-I and IGFBP change in association with reproductive performance, any direct actions that these proteins may have in mediating nutritional effects on the hypothalamic:pituitary:ovarian axis remains to be determined. Circulating concentrations of IGF-I increase during the preovulatory period (Rutter et al., 1989) and concentrations of IGF-I and IGFBP-2 have been shown to be correlated with circulating concentrations of LH during the preovulatory period (Funston et al., 1996). In rats, IGF-I has been shown to cross the blood-brain barrier and localize in the hypothalamus to a greater extent than insulin (Reinhardt and Bondy, 1994). Receptors for IGF-I in the median eminence of rats increase in association with nutrient restriction sufficient to decrease circulating IGF-I (Bohannon et al., 1988). Several IGFBP and IGF-I exist in the hypothalamus, anterior pituitary, and stalk median eminence of cattle (Funston et al., 1993). Insulin-like growth factor I regulates secretion of GH via actions on the hypothalamus and pituitary (Berelowitz et al., 1981; Tannenbaum et al., 1983; Lamberts et al., 1989; Namba et al., 1989) and may also regulate pituitary gonadotrope function (Kanematsu et al., 1991; Atkin et al., 1993; Chandrashekar and Bartke, 1993; Soldani et al., 1995). Profiles of IGFBP in bovine pituitaries change with respect to stage of the estrous cycle (Funston et al., 1995). In vitro treatment of bovine pituitary tissue with LHRH stimulates secretion of IGFBP-2 (Roberts and Funston, 1993). Estrogen increases amounts of mRNA for IGF-I and IGFBP-2 in the rat pituitary (Michels et al., 1993) and levels of protein and mRNA for IGFBP-2 in pituitaries from ovariectomized ewes (Clapper et al., 1996). Collectively, these results indicate that the IGF system may regulate hypothalamic-pituitary function and that the hypophyseal IGF system may be regulated by ovarian and hypothalamic hormones. Thus, circulating and(or) locally produced components of the IGF system may provide a mechanism by which changes in metabolic status of animals may be perceived at the hypothalamic:pituitary:ovarian axis to regulate reproductive function.

Implications

Failure of cows to resume cycling after calving is a major factor influencing the economic viability of the beef cattle industry. The present study describes the associations between the growth hormone:insulin-like growth factor axis and reproductive function in postpartum cattle maintained on levels of dietary intake similar to those in many areas of beef cattle production. Results indicate that clinical analyses of circulating concentrations of growth hormone, insulin-like growth factor I, and insulin-like growth factor binding proteins may predict the nutritional status of postpartum cattle. Applying these results may allow more effective conversion of limited resources to marketable product.

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